experienced by the adhesive bond even when the cell and microvilli are modeled as solid materials. It is further shown that microvillus elasticity plays a role in bond behavior characterization.

3093-Pos

Does Calcium Interact with Titin's Immunoglobulin Domain in Cardiac Muscle?

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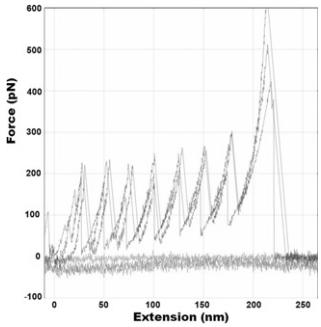
In North America, cardiac muscle diseases such as heart attacks and myopathies are on the rise. Contributing to work in this area, we have focused on a critical muscle protein called titin (connectin). Titin is responsible for all the passive force produced within muscle sarcomeres by acting as a molecular spring preventing muscle over-extension. By adjusting the length of titin's extensible region, a muscle can vary its elastic properties and thus passive force capability. The calcium dependent elasticity of titin has largely been attributed to the PEVK domain, however this mechanism has only been able to explain a tiny contribution of the passive force regulation observed. We propose that other elements in titin, namely the immunoglobulin (Ig) domains, might hold the key to explaining titin's remaining calcium regulated passive force. Fluorescence spectroscopy and atomic force microscopy revealed a change in the microenvironment of the I27 protein with calcium addition.



The application of a mechanical force may trigger the exposure of new binding sites that were buried, therefore Ig domain unfolding may modulate its resting length, elasticity and ligand binding properties, all of which are important for passive force regulation.

3094-Pos

Single-Molecule Kinetics Under Force: Probing Protein Folding and Enzymatic Activity with Optical Tweezers

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Weak non-covalent bonds between and within single molecules govern many aspects of biological structure and function (e.g. receptor-ligand binding, protein folding) In living systems, these interactions are often subject to mechanical forces, which can greatly alter their kinetics and activity. My group develops and applies single-molecule manipulation techniques to explore and quantify these force-dependent kinetics. We have developed a variety of optical tweezers techniques, such as high-resolution 3D position tracking using interference imaging (0.2 nm resolution in z, 1 nm in x-y) [1,2], active feedback for longterm stability in trap height and focus (1-2 nm stability) [2], and intensity modulation imaging for quantifying high-frequency fluctuation above the acquistion rate of a detector (power spectrum measurements above 100 kHz can be made with a slow camera) [3]. We have used these methods to quantify the force-dependent unfolding and refolding kinetics of proteins, including the cytoskeletal protein spectrin in collaboration with E. Evans [4], and the A2 domain of the von Willebrand factor blood clotting protein in collaboration with T. Springer [5]. Furthermore, we have studied the kinetics of the ADAMTS13 enzyme acting on a single A2 domain, and have shown that physiolgical forces in the circulation can act as a cofactor for enzymatic cleavage, regulating hemostatic activity [5].

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3095-Pos

Nanopores as Biosensors: DNA Sequencing and Chiral Discrimination

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Biosensors are stochastic sensors inspired by biology. They are potentially important for many applications in bionanotechnology, from DNA sequencing to single-molecule detection and even chiral discrimination. While the macromolecular properties of the individual components i.e. sensor and analyte are well-characterised, the intricacies of their interaction are less well understood. For full exploitation of biomolecules as stochastic sensors, detailed knowledge of their interactions with other biological and chemical species is desirable. Thus, we have performed a series of molecular dynamics simulations of the bacterial toxin, alpha hemolysin (aHL) and derivative model pores to address issues such as the mechanism of DNA transport through the pore, and the molecular basis of chiral discrimination when the protein is fitted with a molecular adapter (in this case the cyclic molecule, beta cyclodextrin (bCD)).

We study the orientational discrimination of the DNA molecule by restraining the DNA at one end, inside the protein barrel, and applying an electric field. Simulations of the wildtype protein and mutants give good agreement with published experimental data and allow us to explore the molecular basis of discrimination. Our simulations of a model pore (the aHL barrel with only selected sidechains included), allow us to probe the mechanism of DNA threading into the pore once it has already entered the vestibule of the protein. Our results indicate that only key sidechains are required for the interaction with the DNA molecule, and thus have important implications for the future design of engineered protein pores.

Our third set of simulations explores the ability of pores fitted with bCD to discriminate between the enantiomers of ibuprofen. We have used simplified models of pores with full atomistic representation of the bCD and ibuprofen molecules to capture the subtleties of their interaction under an applied external field.

3096-Pos

Modeling of Ionic Currents in a Semiconductor Nanopore

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In this work we are modeling behavior of ionic solution, fully dissociated in water, in the nanopore in a solid-state semiconductor membrane, measuring ionic concentrations and fluxes depending on the voltage applied to the system and geometry of the nanopore. The model is based on the Nernst-Planck and Poisson's equations. Boltzmann statistics is used for charge carrier concentrations in the solution, and Fermi-Dirac statistics is employed to govern electrons and holes concentrations in the semiconductor material. Our approach can be used in modeling semiconductor nanopore membranes with arbitrary internal structure, although the most of results are obtained for a heavily doped n-Si membrane.

3097-Pos

Facilitated Polypeptide Translocation through a Protein Pore

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Facilitated translocation of proteins through a transmembrane protein pore is a ubiquitous and fundamental process in biology. Protein translocation machineries possess various binding sites within the pore interior, but a clear mechanistic understanding of how the interaction of the polypeptides with the binding site alters the underlying kinetics is still missing. Here, we employed standard protein engineering and single-channel electrical recordings to obtain detailed kinetic information of polypeptide translocation through the *staphylococcal* α-hemolysin (αHL) transmembrane pore, a robust, tractable, and versatile β-barrel protein. Binding sites comprised of rings of negatively-charged aspartic acid residues, engineered at different positions within the β barrel, produced significant alterations in the functional features pf the protein pore, facilitating the transport of cationic polypeptides from one side of the membrane to the other. The translocation of polypeptides through the engineered protein pore was dependent on the position of the binding site, the length of the polypeptide as well as its hydrophobic index.

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3098-Pos

Solid-State Nanopore Translocation of Idealized Helical Repeat Proteins

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We report on the translocation of consensus tetratricopeptide repeat (CTPR) proteins with 10 and 20 repeats through single solid-state nanopores formed